American Journal of Hematology 53:158–164 (1996)

Deficiency of Plasma Platelet-Activating Factor Acetylhydrolase: Roles of Blood Cells

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Platelet-activating factor (PAF), a potent mediator of inflammation and circulatory shock, is inactivated by the enzyme PAF acetylhydrolase. Plasma PAF acetylhydrolase deficiency occurs even in healthy subjects. We hypothesized that erythrocyte PAF acetylhydrolase could play a supplementary role in this plasma acetylhydrolase deficiency. We examined 1,030 subjects who participated in mass checkups, and assayed plasma and erythrocyte PAF acetylhydrolase. We also investigated the degradation of exogenous PAF by erythrocytes or other blood cells obtained from subjects who exhibited the plasma enzyme deficiency. The incidence of the plasma enzyme deficiency in this general Japanese population was 4.7% (48/1,030). None of the subjects with the deficiency had a history of allergy, circulatory shock, or chronic inflammatory diseases. The mean values for erythrocyte cytosolic PAF acetylhydrolase activity in the normal and deficient subjects were 0.51 ± 0.15 (SD) and 0.71 ± 0.28 nkat (nmol/s)/g protein, respectively, and the difference was significant ($P < 0.001$, Mann-Whitney U-test). The half-life of 10 nmol/l [^{3}H]PAF in plasma from normal subjects was about 5 min, and the half-life in whole blood or erythrocyte suspension in autologous plasma was almost the same as that in plasma. In plasma from deficient subjects, unchanged PAF virtually remained and the degradation in whole blood or erythrocyte suspension was a little faster than in plasma. We conclude that erythrocytes contribute only little to PAF metabolism in normal blood but they account for almost all of the slow PAF degradation in blood from subjects deficient in plasma PAF acetylhydrolase. © 1996 Wiley-Liss, Inc.

Key words: human erythrocytes, platelet-activating factor, acetylhydrolase, plasma enzyme deficiency, mass checkup

INTRODUCTION

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent bioactive phospholipid [1] that induces platelet and leukocyte aggregation and depresses blood pressure; it is thus regarded as a chemical mediator of inflammation and of circulatory shock. PAF acetylhydrolase (PAF-AH, EC 3.1.1.47) is an acid-labile enzyme that inactivates PAF by hydrolyzing the acetyl group at the *sn*-2 position of the molecule [1]. This enzyme was first purified from human plasma [2] and has been molecularly cloned [3]. The plasma enzyme is secreted by macrophages [3,4], platelets [5], and liver cells [6,7], and is associated with both low- and high-density lipoprotein particles [8]. The injection of mRNA from human macrophages and HepG2 cells into oocytes results in the expression of a PAF-AH indistinguishable from the plasma enzyme [9]. Miwa et al. [10] were the first to describe subjects who had no or extremely low PAF-AH activity in plasma. Inherited deficiency of plasma PAF-AH is the result of a point mutation in the gene [11]. Although an association of inherited PAF-AH deficiency with bronchial asthma was demonstrated [10], most of the deficient subjects were free of any particular diseases. It is still not known why subjects with plasma PAF-AH deficiency are free of the clinical symptoms caused by PAF.

Stafforini et al. [12] demonstrated the presence of PAF-AH in human erythrocytes, and they showed that PAF-AH activity in erythrocytes was biochemically distinct from the activity in plasma, platelets, tissues, and leukocytes

Received for publication June 8, 1995; accepted June 18, 1996.

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[13]. They purified the human erythrocyte cytosolic PAF-AH as a 25-kDa protein [14]. We have described the presence of PAF-AH in erythrocyte membranes [15]. We also found low enzyme activity in the cytosol and membranes of erythrocytes obtained from patients with cerebral thrombosis [16,17]; this low enzyme activity was associated with higher levels of blood PAF (or PAF-like lipids) [18] and plasma PAF-AH [19]. The activity of erythrocyte PAF-AH decreases with cell aging, as does membrane fluidity, and it appears that erythrocyte PAF-AH may play an important role in repairing membrane phospholipids during the finite life-span of the cells [20]. These findings suggest that erythrocyte PAF-AH may be effective in inactivating both cellular and extracellular PAF and PAF-like lipids.

Against this background, we hypothesized that erythrocyte PAF-AH could compensate for the deficient plasma enzyme, and that it is because of this erythrocyte activity that most subjects with the plasma enzyme deficiency maintain their health. To address this hypothesis, we examined 1,030 participants in health checkups, and assayed plasma and erythrocyte PAF-AH. We also examined the inactivation of exogenous PAF by erythrocytes or other blood cells obtained from subjects with the plasma PAF-AH deficiency.

MATERIALS AND METHODS

Reagents

Radiolabeled PAF (1-*O*-hexadecyl-[2-*acetyl*-^{3}H]-*sn*-glycero-3-phosphocholine, 370 GBq/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled PAF, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and human serum albumin (HSA) were from Sigma Chemical Co. Ltd. (St. Louis, MO). Bovine serum albumin (BSA) was from Armour Pharmaceutical Co. (Chicago, IL). Ficoll-Paque was from Pharmacia LKB (Uppsala, Sweden). RPMI Medium 1640 (GIBCO BRL, Gaithersburg, MD) was from Life Technologies, Inc. (Grand Island, NY). Fetal bovine serum (FBS) was from JRH Biosciences (Lenexa, KS). A cocktail for liquid scintillation (Clear-sol I) was from Nacalai Tesque, Inc. (Kyoto, Japan). Other chemicals used were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Subjects

The subjects studied were 1,030 participants in a health checkup program (781 women and 249 men), who ranged in age from 34 to 91 years (mean ± SD, 63.2 ± 8.3 years). They resided in Hirosaki City, Japan, where annual checkups are carried out under the auspices of our laboratory. Physical examination (including blood pressure), urinalysis, electrocardiogram, and biochemical and hematological screenings were performed in all subjects.

Blood Samples

After obtaining their informed consent, we withdrew fasting blood samples from the antecubital vein and mixed the samples with 5.5% (v/v) of 77 mmol/l disodium ethylenediaminetetraacetate (EDTA) solution. A membrane-free hemolysate (cytosol) of erythrocytes was prepared, as described previously [20]. Briefly, the erythrocytes were washed three times with 10 volumes of 20 mmol/l phosphate-buffered saline (PBS; pH 7.4), and lysed by mixing with 20 volumes of 7 mmol/l sodium phosphate buffer (pH 7.4) at 4°C for 20 min. After centrifugation at 20,000*g* for 20 min, membrane-free cytosol was recovered. The total protein content was determined by the method of Lowry et al. [21], with BSA as standard. Blood samples were also anticoagulated with 1% (v/v) of 1×10^6 U/l sodium heparin for the preparation of platelet-rich plasma (PRP) or erythrocyte suspension in autologous plasma.

PAF-AH Assay for Plasma and Erythrocyte Cytosol

The PAF-AH activity in plasma and the erythrocyte cytosol was assayed according to the method of Stafforini et al. [2]. Briefly, the specimen was incubated at 37°C for 30 min with 80 μmol/l [2-*acetyl*-^{3}H]PAF, adjusted to 833 Bq (5.0×10^4dpm)/nmol by adding unlabelled PAF and dissolved by sonication in 90 mmol/l HEPES buffer (pH 7.4); undegraded [2-*acetyl*-^{3}H]PAF was then removed by applying the assay mixture to an octadecylsilica gel cartridge (Easy Chromato C18; Kurabo Biomedical Company, Osaka, Japan), and the radioactivity of liberated [^{3}H]acetate in the washes, mixed with Clear-sol I, was then measured by a liquid scintillation counter (LSC-3500; Aloka Co. Ltd., Tokyo, Japan). The details of this method have been described elsewhere [20]. In preliminary experiments, the erythrocyte PAF-AH activity was found to be sensitive to trypsin and NaF [15,16], and was shown to be different from the activity of the intracellular enzyme derived from leukocytes [13] and the extracellular type derived from platelets [5].

Half-Life Assay of PAF in Blood

The half-life assay of exogenous PAF in whole blood, PRP (adjusted to 2.6×10^{11} cells/l), platelet-poor plasma (PPP), or 50% (v/v) erythrocyte suspension in autologous PPP was carried out by the method of Stafforini et al. [22]. Briefly, 1.0 ml of the specimen was incubated with 10 nmol/l [2-*acetyl*-^{3}H]PAF (262.7 Bq [1.576×10^4 dpm]/pmol) at 37°C for various periods of time (1–16 min) with gentle shaking. After incubation, aliquots (100–200 μl) were pipetted out and the lipids were then extracted and fractionated by thin layer chromatography. The area of silica gel containing PAF was scraped and the radioactivity measured.

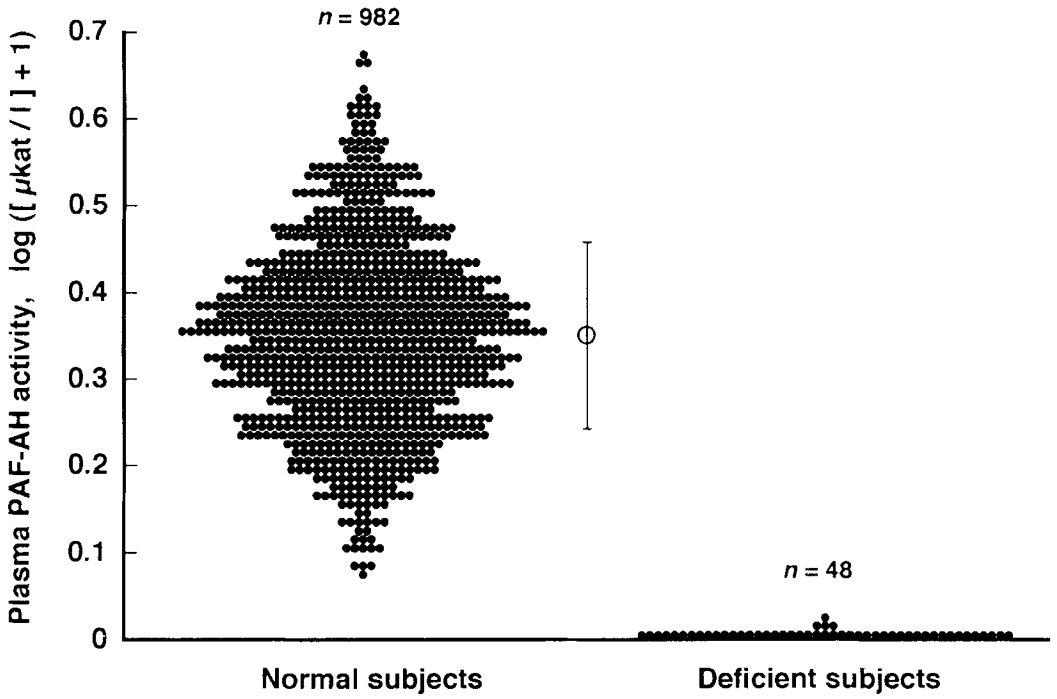

Fig. 1. Plots of platelet-activating factor acetylhydrolase (PAF-AH) activity in plasma from 1,030 subjects. The data were log-transformed to normalize the distribution. The subjects were divided into two groups: the normal group, in whom plasma PAF-AH activity exceeded 0.17 μkat/l (10 nmol ml^{-1} min^{-1}), and the deficient group, in whom the activity was virtually zero (less than 0.07 μkat/l). The mean value in the normal subjects, 1.24 μkat/l, is indicated by the open circle, with the vertical bar showing SD (95% confidence interval, 0.38–2.66 μkat/l).

PAF-AH Activity of Macrophages

Peripheral blood monocytes were isolated and the monocyte-derived macrophages were cultured according to Elstad et al. [23]. Briefly, mononuclear leukocytes were isolated from blood by density gradient centrifugation using Ficoll-Paque. Then, monocyte-rich suspension was placed into a 35-mm tissue culture dish (Falcon Oxnard, Lincoln Park, NJ) and incubated for 1 hr at 37°C to allow adherence. After removal of the nonadherent cells by aspiration, the adherent cells were cultured in 1.0 ml of RPMI Medium 1640 (supplemented with 10% [v/v] FBS) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Macrophages cultured in this manner exhibited characteristic morphology, as assesses by phase contrast microscopy. After 1, 2, and 7 days of culture, 24-hr conditioned medium was recovered and the cells were washed, scraped from the tissue culture plate in 1.0 ml of 20 mmol/l PBS (pH 7.4, 4°C), and homogenized by 25 strokes with a Dounce homogenizer (Wheaton Scientific, Millville, NJ). PAF-AH activity in the conditioned medium or the homogenate was determined in a similar manner. To assess the possible contribution of Ca^{2+}-dependent phospholipase A_2, the assay was performed in the presence or absence of 5 mmol/l $CaCl_2$.

Statistics

Values are expressed as means ± SD. Statistical significance was tested by Student's or Welch's *t*-test, the χ^2 test, or the Mann-Whitney U-test. All probability (*P*) values were based on two-tailed tests.

RESULTS

The plots of plasma PAF-AH activity in all subjects are shown in Figure 1. The subjects were divided into two groups: normal subjects, whose enzyme activity exceeded 0.17 μkat/l (10 nmol ml^{-1} min^{-1}), and deficient subjects, whose enzyme activity was virtually zero (less than 0.07 μkat/l). The mean value in the normal subjects was 1.24 (95% confidence interval, 0.38–2.66) μkat/l. The incidence of plasma enzyme deficiency was 4.7% (48/1,030).

No significant differences between the normal and deficient subjects were found in the percentages of smokers, the results of blood screening tests, and the incidence of abnormal findings in urinalysis or electrocardiogram. We identified various past and present diseases including hypertension, gastrointestinal disease, heart disease, hyperlipidemia, liver disease, stroke, and diabetes mellitus; however, there was no significant difference in the inci-

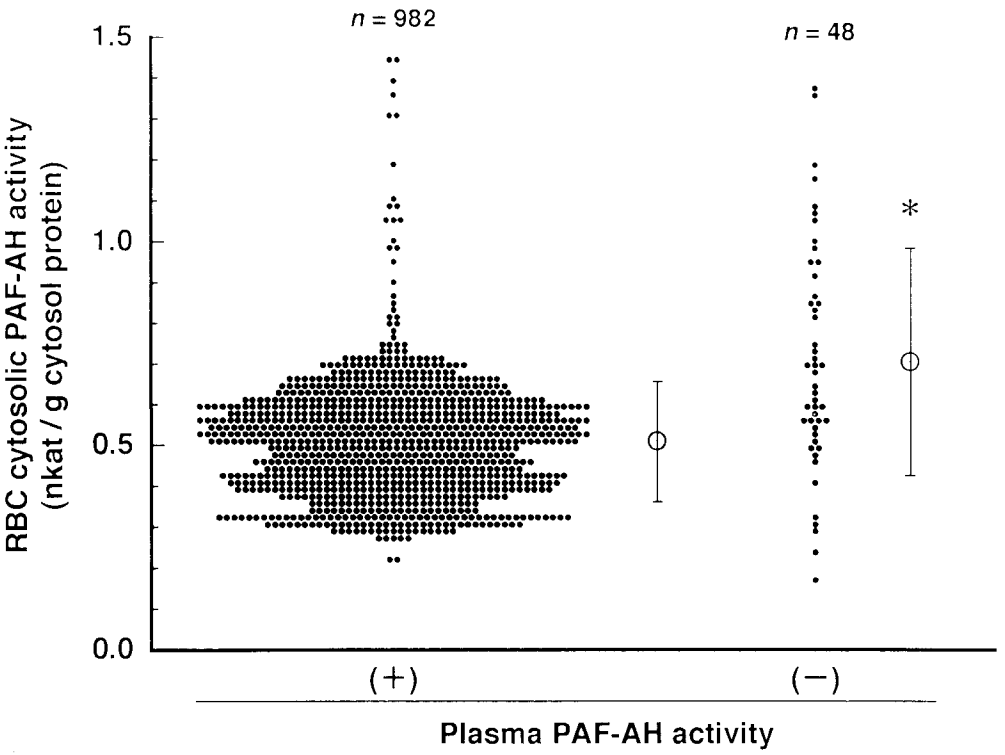

Fig. 2. Plots of platelet-activating factor acetylhydrolase (PAF-AH) activity in the cytosol of erythrocytes (RBCs) from 1,030 subjects with normal and deficient plasma PAF-AH activity. The mean values are indicated by open circles, with vertical bars showing SD. *$P < 0.001$ (Mann-Whitney U-test) vs. the value in subjects with normal plasma PAF-AH activity.

dence of these diseases between the two groups. Moreover, no subjects with the deficiency had a history of allergy, circulatory shock, or chronic inflammatory diseases.

Erythrocyte cytosolic PAF-AH activity in the two groups is plotted in Figure 2. The values for the erythrocyte activity in the normal and plasma PAF-AH deficient subjects were 0.51 ± 0.15 and 0.71 ± 0.28 nkat/g cytosol protein, respectively, and the difference was significant ($P < 0.001$, Mann-Whitney U-test). The levels of cytosol protein in the two groups were the same.

To assess the effect of blood cells on the inactivation of PAF in plasma PAF-AH deficient subjects, we examined the metabolism of exogenous PAF in blood or its components including erythrocytes (Fig. 3). In normal subjects, whole blood, PRP (2.6×10^{11} cells/l), or 50% (v/v) erythrocyte suspension in autologous PPP metabolized PAF at a rate similar to PPP, implying plasma PAF-AH accounted for most of PAF-degrading activity in blood. In the deficient subjects, the degradation of PAF in both PRP and PPP was no more than natural decay; and whole blood or erythrocyte suspension metabolized PAF faster, although a little, than plasma itself.

Figure 4 shows the results of PAF-AH activity in cultured monocyte-derived macrophages isolated from peripheral blood of normal or deficient subjects. Cultured macrophages from normal subjects released PAF-AH activity into the medium until 7 days in culture but not the cells from deficient subjects. Similarly, the normal cells exhibited intracellular PAF-AH activity while the deficient cells lacked such activity. All of these activities were not enhanced by the addition of Ca^{2+} (5 mmol/l, data not shown).

DISCUSSION

In this study, we found that in mass medical examinations in a Japanese population, the incidence of subjects with deficiency of plasma PAF-AH activity was 4.7% (48/1,030). This incidence was similar to the incidence of 3.9% (32/816) reported by Miwa et al. [10] in healthy adults. In their analysis of five Japanese families, they found that the deficiency appeared to be transmitted by autosomal recessive heredity; they also found that the incidence was significantly higher (12%, 5/42) in asthmatic children with moderate and severe respiratory symptoms [10]. However, the precise nature and the pathophysiological significance of this deficiency is not known.

We evaluated 48 subjects with plasma PAF-AH deficiency. The deficient subjects did not seem to be predis-

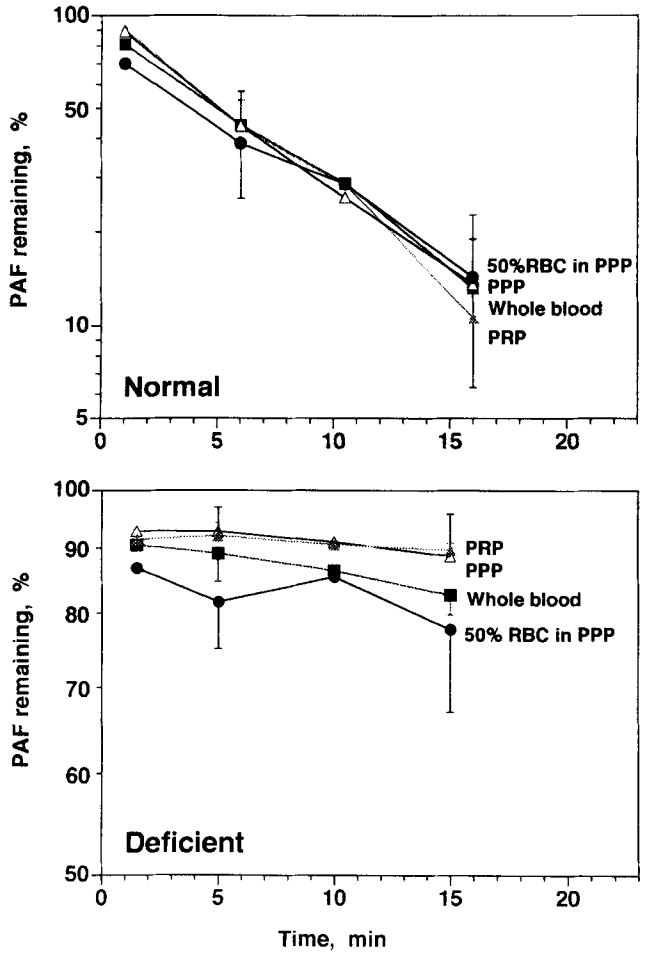

Fig. 3. The metabolism of platelet-activating factor (PAF) in blood or its components from normal subjects with plasma PAF acetylhydrolase deficiency. We added [*2-acetyl*-^{3}H]PAF (10 nmol/l) to whole blood, platelet-rich plasma (PRP, 2.6 × 10^{11} cells/l), platelet-poor plasma (PPP), or 50% (v/v) erythrocyte (RBC) suspension in autologous PPP. Aliquotes were pipetted at the times shown, and remaining [*2-acetyl*-^{3}H]PAF was extracted and separated by thin layer chromatography, and quantified by liquid scintillation counting. The data shown are representative or mean values, with vertical bars showing SD of three normal subjects and three deficient subjects.

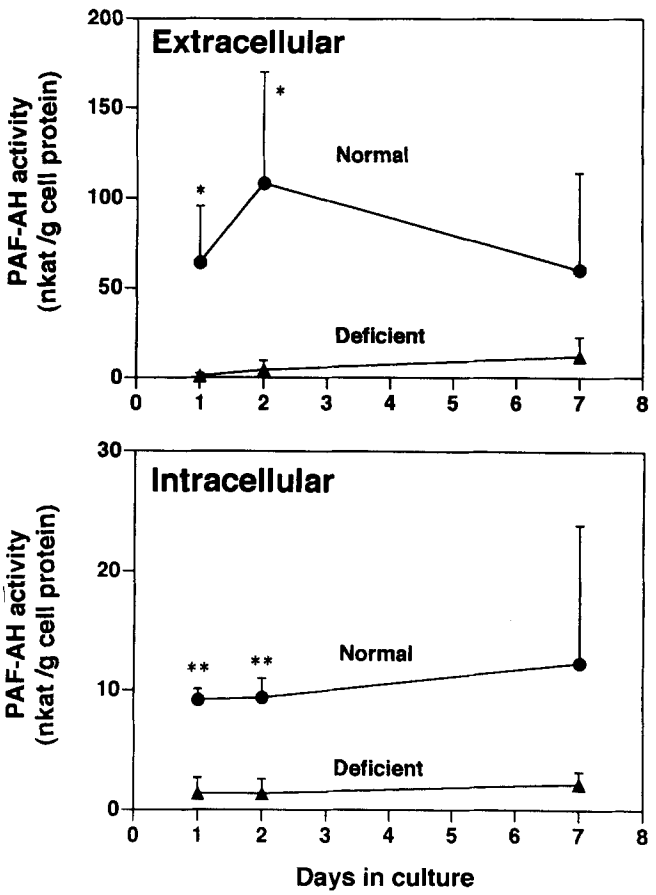

Fig. 4. Production and secretion of platelet-activating factor acetylhydrolase (PAF-AH) by peripheral blood monocyte-derived macrophages from normal subjects or subjects with plasma PAF-AH deficiency. To measure PAF-AH activity secreted from macrophages (extracellular), the conditioned medium was assayed. To measure cell-associated PAF-AH activity (intracellular), the cells were washed, scraped from the culture plate, homogenized, and assayed. The results shown are mean values, with vertical bars showing SD, of three normal subjects and deficient subjects. $^*P < 0.05$ (Welch's *t*-test); $^{**}P < 0.01$ (Student's *t*-test): vs. the values in subjects with plasma PAF-AH deficiency. These activities were not enhanced by the addition of Ca^{2+} (5 mmol/l, data not shown).

posed to any particular diseases compared to normal subjects, and they did not exhibit the clinical conditions, including allergic or inflammatory disorders, that could be directly related to increased PAF levels [1]. Most of the deficient subjects appeared to have normal results in laboratory screenings.

In healthy persons, the level of plasma PAF-AH activity increases with advancing age [24]. However, in our subjects, the PAF-AH deficiency was not related to age: the age distribution of the deficient subjects was the same as that of the controls. Plasma PAF-AH activity is lowered by the administration of estrogenic hormone [25] and by cigarette smoke extract [26]. However, our subjects did not have any symptoms related to the excess production of such hormones. The effect of smoking could also be excluded since we found that the activity was higher in healthy habitual cigarette smokers than in healthy nonsmokers in our previous study [27].

Plasma PAF-AH activity is reported to be low in patients with diseases such as systemic lupus erythematosus [28], neonatal necrotizing enterocolitis [29], severe coronary artery disease [30], acute myocardial infarction [31], and sepsis [32] (although the activity was higher in patients with sepsis in whom the outcome was fatal than in those in whom the outcome was not fatal [33]). However, the magnitude of the decrease in these diseases is

far less than the complete absence of the activity found in PAF-AH deficiency. In the present study, there was no evidence for the association of plasma PAF-AH deficiency with any particular diseases, as well.

Phospholipase A_2 and lecithin-cholesterol acyltransferase also exhibit PAF-hydrolyzing activity [32–34]. In subjects with plasma PAF-AH deficiency, these enzymes could thus be the candidates for factors that replace PAF-AH. However, we did not detect [^{3}H]acetate released from [2-*acetyl*-^{3}H]PAF in the plasma samples of the 48 PAF-AH deficient subjects, indicating that these two enzymes do not contribute to PAF-hydrolyzing activity in plasma PAF-AH deficiency.

Erythrocytes contain PAF-AH in both their cytosol [12–14,16,17,20] and membranes [14,15,17,20]. Because PAF is a phospholipid, it can be taken up by intact cells through a receptor-independent mechanism [35]; and cytosolic enzyme may be potentially effective in degrading extracellular PAF. None of our subjects, neither those with or without plasma PAF-AH deficiency, exhibited a deficiency of erythrocyte PAF-AH activity. Intriguingly, the mean value for erythrocyte PAF-AH activity was higher in the subjects with plasma PAF-AH deficiency than in the normal subjects. Although the mechanism of this difference was unclear, the higher erythrocyte PAF-AH activity may favor the inactivation of PAF in the circulation. This finding might, as it were, show an aspect of adaptation.

In the light of these findings, we hypothesized that the presence of erythrocyte PAF-AH activity could protect subjects with plasma PAF-AH deficiency from serious clinical problems. As shown in the present study, the erythrocytes of the deficient subjects had normal or higher levels of cytosolic PAF-AH activity. Although there may not be any additional effect of erythrocytes on PAF-inactivation in normal subjects, they may constitute the sole route for metabolizing PAF in blood of plasma PAF-AH deficiency. In this regard, Stafforini et al. [12–14] also proposed that the hemolysis of erythrocytes at sites of inflammation and the subsequent release of PAF-AH could down-regulate the responses elicited by PAF.

Macrophages [3,4], platelets [5], and liver cells [6,7] are regarded as sources of the plasma form of PAF-AH. Since plasma PAF-AH deficiency is the result of a point mutation in the gene [11], these sources are also thought to be deficient in the enzyme activity. Indeed, cultured macrophages of the deficient subjects did not exhibit extracellular nor intracellular PAF-AH activity. Although the involvement of Ca^{2+}-dependent phospholipases is unlikely in the present study, the assay condition may have been inadequate for such enzymes. Platelets of the deficient subjects did not also exhibit a substantial level of PAF-degrading activity.

In conclusion, plasma PAF-AH accounts for almost all of the PAF-degradation in blood from normal subjects; whereas in plasma PAF-AH deficiency, the enzyme in erythrocytes contributes virtually exclusively to the metabolism of PAF in blood, which was much slower than normal.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (06680674, 07672482). We thank Dr. Mutsu Takamatsu, Mr. Hajime Akitaya, Ms. Makiko Takahashi, and the staff of the Checkup Center of Adult Diseases, Hirosaki City Medical Association, for their support and technical help, and Ms. Kumiko Satoh for help in preparing the manuscript.

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